in the cytoplasmic side of the transmembrane domain of integrin α- and β-subunits named as the inner membrane clasp, a hydrophobic packing of a few transmembrane residues on extracellular side between α- and β-subunits that is termed as the outer membrane clasp, and the key interaction group of βA domain and βTD domain. Molecular details of this key interaction group as well as events that lead to detachment of βTD and βA domains have remained ambiguous.
Full-length structure of integrin αIIbβ3 embedded in a patch of lipid bilayer was used to simulate its interactions with three soluble RGD ligands as well as talin, using a molecular dynamics software. We showed that talin's interaction with the membrane-proximal and membrane-distal regions of integrin cytoplasmic-transmembrane domains significantly loosens the inner membrane clasp as well as an additional salt-bridge (R734-E1006), which facilitates integrin activation through the separation of integrin's α- and β-subunits. Also, it is shown that interaction of the Arg of the RGD sequence may activate integrin via disrupting the key interaction group between Lys346 on βA domain and Ser663/Ser664 on the βTD. Interestingly, we observed the full dissociation of βA and βTd domains when this interaction group was disrupted and was eventually dissociated as a result of a competition between the Arg of the RGD peptide with Ser664. Consequently, we proposed a mechanistic scenario as a potential mechanism for outside-in activation of integrin αIIbβ3 by soluble RGD ligand that reconciles the switchblade and dead-bolt models for integrin activation.

1209-Plat
Single Molecule Imaging of Human Epidermal Growth Factor Receptors
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The human epidermal growth factor receptor (HER/ErbB) family of receptor tyrosine kinases encompasses transmembrane signaling proteins important for cellular growth, differentiation, and survival. The four members of this family (EGFR, HER2, HER4 and the HER3 pseudokinase receptor) are able to bind to numerous different growth factor ligands, leading to their homo and hetero-oligomerization and subsequent activation. This combinatorial potential gives rise to a diverse signaling output, which is strongly affected by misregulation of any one receptor. For example, mutations in EGFR and HER2 overexpression are primary mechanisms driving lung and breast cancer, respectively. Under these conditions, heterodimerization of these receptors with the HER3 pseudokinase confers resistance to tumors treated with EGFR and HER2-targeted therapeutics. The molecular basis for these heterodimeric interactions and their regulation by growth factors remains poorly understood. HER receptor heterodimers have never been observed directly, and their existence remain inferred from the analysis of downstream signaling. To understand the scope and specificity of heterodimeric interactions between the HER3 pseudokinase receptor and its active HER homologs, we seek to directly investigate the underlying receptor activation mechanisms using high resolution fluorescence microscopy. We quantify the extent of heterodimeric interactions in response to ligand binding using both live cell single molecule tracking and stochastic optical reconstruction microscopy (STORM). These techniques allow us to probe the timescales of receptor interactions as a function of ligand binding, the dependence on receptor density at the membrane surface, and the specificity of heterodimer formation. This work aims to contribute to the fundamental understanding of the activation mechanism of HER receptors and to help advance development of new therapeutics targeting aberrant cross-talk among HER receptors in human disease.

Platform: Structure and Dynamics of RNA in Biology

1210-Plat
Deciphering Ribosomal Frameshifting Dynamics
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Ribosomes programmed by specific messenger RNA (mRNA) sequence elements can switch translation reading frames and synthesize different polypeptides from a single template. The *Escherichia coli dnaX* mRNA encodes two DNA polymerase III subunits, τ and γ, synthesized from 0-frame and probabilistic −1-slip across the slippery sequence: AAAAAAG. When further enhanced by structural barriers situated around the slippery sequence-an internal Shine-Dalgarno sequence and a stable hairpin stem loop, an 80% (= γ/(γ+τ)) frameshift efficiency is attained. Here, we attempt to determine the frameshift timing within one translation cycle by following a single ribosome translating a frameshift-promoting mRNA held on optical tweezers. In parallel, by mass spectrometry (MS), we survey the synthesized polypeptides to resolve where on the mRNA the ribosome has slipped.
From the mass spectra of polypeptides terminated at the −1-frame stop codon, we learned that the ribosome −1-slips from more than one codon position around the slippery sequence. Some −1-frameshifted polypeptides were found to bear an extra amino acid, or to lack one, indicating that slipping sizes of −4 and +2-nt also occurred. Similarly, distinctive large-scale fluctuating translocation dynamics were seen in our real-time single-ribosome translation trajectories. This reveals that a translocating ribosome can explore a broad range of frameshift pathways. Frequently adopted frameshift pathways, i.e. the more abundant frameshifted species resolved by MS, exhibit a preference for minimizing codon:anticodon base-pair mismatches on the ribosome after a slip. Mismatch-containing ribosomes can be prematurely terminated by release factors, resulting in release of incomplete peptides. Indeed, we observed higher yields of incomplete peptides that are terminated at frameshift sites where significant mismatches were encountered. These species coincide with the prematurely stalled ribosomes recorded in the translation trajectories. Collectively what emerges from our results is a versatile ribosomal frameshifting scheme during mRNA translocation, facilitating branching of frameshift pathways.

1211-Plat
Unraveling the Mystery of Ribosome Induced RNA Unfolding
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During translation, the ribosome encounters various structures in the mRNA from simple hairpins to more complex tertiary RNA folds. These structures must be unwound for accurate decoding and translation to proceed. To probe the unwinding activity of the ribosome, we prepared dye labeled RNA transcripts and dye labeled ribosomes to directly report on the folded/unfolded states of the RNA at various stages along the translational elongation cycle. Consistent with previous studies, we found that the ribosome was sufficient to resolve RNA structures in the absence of any additional factors. Surprisingly, significant unwinding was observed in the absence of translocation or motion of the ribosome along the mRNA and can be attributed to dynamic motions within and between the two ribosomal subunits. This suggests that thermal energy resident within these ribosomal motions is sufficient to lower the energetic barrier of unfolding. The extent of unwinding was observed to depend on the relative stability of the RNA structure used and the number of tRNAs present in the ribosome. Differences between unfolding of these RNA structures in the presence and absence of the ribosome will be discussed.

1212-Plat
Single-Molecule Profiling of Ribosome Translational Phenomena
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Translation elongation is a heterogeneous process, involving multiple compositional factors stochastically binding to the ribosome to direct protein synthesis, which in turn regulates the conformation of the ribosome. The rate of translation is often regulated by the underlying messenger RNA (mRNA) sequence and structure. Here, we use single-molecule fluorescence resonant energy transfer (FRET) and colocalization with zero-mode waveguides (ZMWs) to correlate directly ribosome conformations and compositions of thousands of ribosomes simultaneously during multiple rounds of elongation. This allows us to profile global translational rates while delineating mechanistic details of the dynamics with codon resolution. We first studied translation of a canonical mRNA with uniform translation rates to establish the tight interplay between compositional factors and conformational dynamics of the ribosome during elongation. We then determined how mRNA sequences and structures, such as hairpins and possible ribosome-mRNA base pairing, as expressed in the the dnaX −1 frameshifting sequence, perturb the basal elongation process. Our results show how mRNAs can modulate and uncouple ribosomal conformational and compositional dynamics.

1213-Plat
The Ribosome Uses Cooperative Conformational Changes to Maximize the Efficiency of Protein Synthesis
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Execution of individual steps in the multi-step functional cycles of large, multi-component molecular machines such as the ribosome almost universally